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Two New Records of Wing-reduced Tipulidae from North America

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We present two distribution records, COI sequence data, and photographs for the rare brachypterous species *Tipula* (*Vestiplex*) *aldrichiana* and *Tricyphona subaptera*, noting in particular a range expansion into Canada for *T.* (*V.*) *aldrichiana.*

Brachyptery (loss or reduction of flight wings) is associated with range size reduction and geographical isolation and is frequently associated with a high degree of local endemism in montane insect species (Kavanaugh 1985; McCulloch et al. 2016). Other flightless alpine insects are known to comprise cryptic species complexes even within mountain ranges (Schoville and Roderick 2010), largely reflecting the physical separation of alpine habitats, the strong topographic relief limiting dispersal, and the history of glacial events that subdivide contiguous mountain ranges, all leading to geographical isolation. Various hypotheses about the origin of flightless species in such environments, as well as about the role of dispersal after wing reduction versus *in situ* repeated evolution of brachyptery (Medeiros et al. 2015), can be tested once groups that display wing-reduction have been adequately sampled.

Here we report recent collections and a range expansion for two species of Tipulidae that display brachyptery, as well as the first published photographs and genetic data for either of these species. Although many wing-reduced species are known from several genera of Tipulidae (Byers 1969), many of them, especially those that inhabit remote areas where collection is difficult, have not been collected for decades. Therefore, these specimens might provide valuable taxonomic material for future studies on the biogeography of these groups and for the study of wing-reduction in general.

Methods

Specimens were collected by hand into vials containing 100% Ethanol, during surveys of rocky alpine habitats. Digital photographs of adults were taken with a Canon EOS-1D Mark II camera using a Microptics Digital Imaging System. DNA was extracted from the legs of specimens using the standard protocol described in Qiagen's (Valencia, CA, USA) DNeasy kits. The "barcoding" segment of the mitochondrial (mtDNA) protein coding gene COI was amplified using the primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') and the following thermal profile: 2 min at 94°C; 34 cycles of 94°C for 1 min, 51°C for 1 min, and 72°C for 2 min; 12 min at 72°C. PCR product was purified using ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) and then sequenced on an

Applied Biosystems (Grand Island, NY, USA) 3730 DNA Analyzer. Using mitochondrial COI sequences available in Genbank at time of publication, we used BLASTN (Altschul et al. 1990) to report the most genetically similar species with maximal coverage for this region of COI.

Results and Discussion

Taxonomy

I. *Tipula* (*Vestiplex*) *aldrichiana* Alexander 1929. Canada: British Colombia, Cassiar, N59.2486, W129.9095 (WGS84). 1 ♀, 8 Aug. 2014, 23:30–02:00 hrs., S.D. Schoville and M.J. Medeiros. Specimen code SDS14–562; Genbank accession code KY114055.

The collection of *T. aldrichiana* occurred at night, in a rocky habitat near a creek draining snowmelt (air temperature was just above freezing). The specimen (Fig. 1) was found walking slowly on a small snow-patch. Other insects foraging in the same habitat included *Grylloblatta campodeiformis nahanni* Kamp 1979, omaline rove beetles (Staphylinidae), and *Nebria* (*Boreonebria*) spp. (Carabidae). Specimen deposited in the California Academy of Sciences (CASENT 8125472).

Tipula (*Vestiplex*) *aldrichiana* has flightless and brachypterous females, although the males are fully winged. This species is previously known from Alaska and several areas of Russia, but has not been previously recorded in Canada. The last collection of this species occurred in 1958, in East Beringia, Russia (Starkevich and Paramonov 2016).

II. *Tricyphona subaptera* (Alexander 1917). USA: California: Ansel Adams Wilderness, Mono Co., East Donohue Pass, 37.7615°N, 119.2394°W (WGS84). 2 ♂, 17 July 2016, 13:00–14:00 hrs., S.D. Schoville and B.A. Pieper. Specimen code SDS16-335; Genbank accession code KY114056. Specimens deposited in the California Academy of Sciences (CASENT 8125473 and CASENT 81254754, respectively).

The collection of *T. subaptera* occurred at mid-day, in a grassy alpine meadow near a small creek (the air temperature was about 22–25°C). Talus habitat otherwise dominates the locality. The specimens, one of which is shown in Fig. 2, were found next to one another, walking in the grass, about two meters from the creek edge.

Tricyphona subaptera displays brachyptery in both sexes. Although this species has previously been collected from Yosemite National Park and nearby areas, the most recent of the earlier collections was made in 1957.

COI data

For *Tipula* (*Vestiplex*) *aldrichiana*, there is a 5% sequence divergence with the most similar sequence (KR756861) belonging to *Tipula* (*Vestiplex*) *balioptera*; there is 100% coverage between these two sequences. This fully winged species is known from Eastern Russia and is widespread in Canada and the northern United States (Alexander 1966). For *Tricyphona subaptera*, there is 10% sequence divergence as compared to *Tricyphona calcar*, but this sequence has only 96% coverage (KM571857) in common with our *Tricyphona subaptera* sequence. *Tricyphona calcar*, a fully winged species, is known from the eastern US and Canada (Alexander 1966). Due to very incomplete sampling of putative close relatives, attempting to present a hypothesis of phylogenetic relationships of these species to other Tipulidae would not be prudent at this time.

Concluding comments

Flightless insects can provide important information about species richness and endemism in a region, serving as useful surrogates for community-wide conservation priorities (Moritz et al.

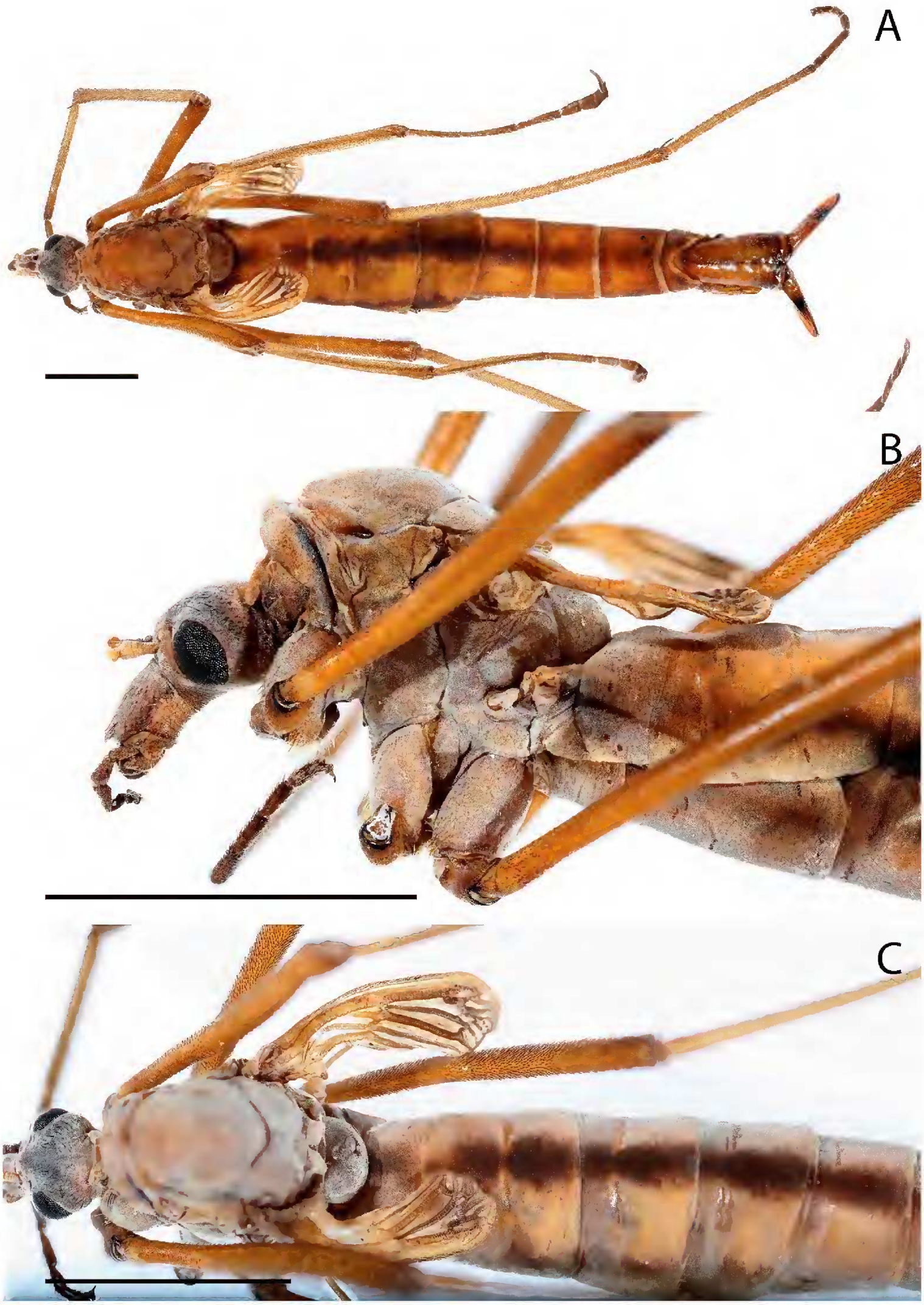

FIGURE 1. *Tipula* (*Vestiplex*) *aldrichiana* Alexander. A: Dorsal view. B: Left lateral view of head and thorax. C: Dorsal view showing wing morphology. Scale bars = 2 mm.

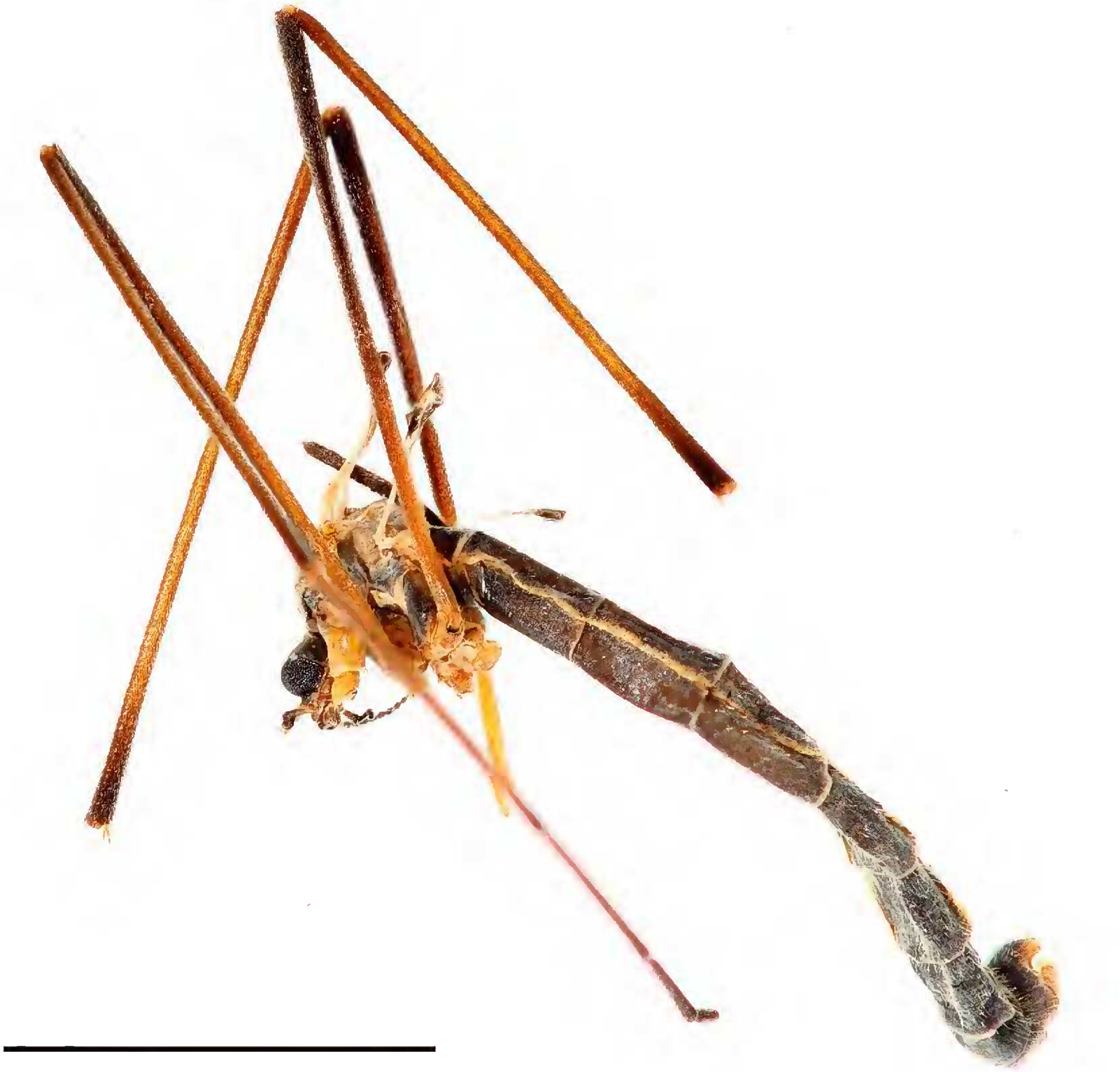

FIGURE 2. *Tricyphona subaptera* (Alexander). Left lateral view. Scale bar = 5 mm.

2001; Matenaar et al. 2015). Additionally, flightless insects offer the opportunity to test hypotheses regarding the ecological conditions and life-history traits most likely to lead to flight loss, but few groups with flightless species have been sampled adequately, a necessary first step in this process (Roff 1994). The lack of current knowledge of the distribution and diversity of flightless insects, including tipulids, is an unfortunate short-coming that needs to be addressed, and it is our hope that the records and information presented here are of use in future revisions of these large genera (*Tipula* subg. *Vestiplex* includes 214 known species; *Tricyphona* includes 130 species).

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